EFFECT OF *Aegle marmelos* AND *Andrographis paniculata* ON HAEMATOLOGY AND SERUM BIOCHEMISTRY IN AFLATOXICOSIS OF BROILER CHICKEN

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Recieved: 13-07-2019 Accepted 21-08-2019

ABSTRACT

The present study was undertaken to find out the effect of *Aegle marmelos* and *Andrographis paniculata* on haematology and serum biochemistry in aflatoxicosis of broiler chicken. Day old broiler chicken were divided into eight groups and fed with different diets ranging from T1 to T8. Haemoglobin values of T3, T4, T5, T6, T6, T7 and T8 were statistically similar to that of control (T1). Serum total protein of broiler chicks of T3, T4, T5, T6, T7 and T8 were higher than T2 and were comparable to that of T1. Broiler chicks of T3, T4, T5, T6, T7 and T8 showed statistically similar BUN values as that of T1. The enzyme values returned to normal levels as that of control in rest of the treatments at 14 and 21 days of the experiment.

Key words: *Aegle marmelos, Aflatoxicosis, Andrographis paniculata*

INTRODUCTION

Aflatoxins (AF) are produced by toxigenic strains of the fungi, *Aspergillus flavus* and *A. parasiticus* in food grains. These fungi invade and grow in feed ingredients and feed under favourable environments especially under hot humid conditions to produce their secondary metabolites. Introduction of strict control measures has brought the level of contamination of the toxin in feed under permissible limit. The

objective of this study was to evaluate the ameliorative effect of *Aegle marmelos and Andrographis paniculata*, two well-known plants with hepatoprotective activities on haematology and serum biochemistry among broiler chicken on aflatoxicosis.

MATERIALS AND METHODS

A. flavus NRRL 6513 culture was subcultured every 15 days and maintained at room temperature to ensure availability of fresh spores. Maize was used as substrate for producing aflatoxins (Shotwell *et al*., 1966). Aflatoxin B1 content was estimated as 63.77 ppm using TLC method at AFAQAL Namakkal. *A. marmelos* mature leaves and *A. paniculata* whole plants were collected locally and were authenticated. The plant materials were dried under shade and powdered using a pulverizer. Forty-eight numbers of day-old Vencobb 400 strain broiler chicks procured locally, which were randomly divided into eight groups of six birds each and fed with the following diets for 21 days viz. standard feed (T1), standard feed with 100 ppb aflatoxin(AF) (T2), standard feed with 0.10 per cent *A. marmelos* powder and 0.10 per cent *A. paniculata* powder (T3), standard feed with 100ppb AF and 0.10 per cent *A. marmelos* powder (T4), standard feed with 100ppb AF and 0.10 per cent *A. paniculata* powder (T5), standard feed with 100 ppb AF and 0.20 per cent *A. marmelos* powder (T6), standard feed with 100ppb AF and 0.20 per cent *A. paniculata* powder (T7) and standard feed containing 100 ppb AF, 0.10 per cent *A. marmelos* powder and 0.10 per cent *A. paniculata* powder (T8). Blood was collected from the wing vein of all the birds at day 7 and 14 for enzymology and on day 21 for haematological and serum biochemical analysis. Data on different parameters were analysed statistically using SPSS version 24.0.

RESULTS

The haematological parameters recorded at 21st day experiment are presented in Table 1.

Serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) values were recorded at day 7, 14 and 21 of experiment. Total protein, albumin, globulin, BUN and creatinine values were observed at 21st day of experiment. Mean (±SE) total protein, albumin, globulin and A: G ratio, blood urea nitrogen (BUN) and creatinine levels of all treatment birds recorded at 21st day are presented in Table 2.

Table 1. Mean (±SE) haematological parameters broiler chicks

GROUPS	Hb (g/dl)	VPRC (%)	TLC(10^9/L)
T1	9.67 ± 0.24^{a}	28.13 ± 0.76^{a}	131.15 ± 1.20^{a}
T2	8.37 ± 0.14^{b}	23.12 ± 0.67^{bc}	120.55 ± 1.37^{d}
T3	9.25 ± 0.19^{a}	24.78 ± 0.46^{b}	124.55 ± 1.23^{cd}
T4	9.37 ± 0.37^{a}	24.42 ± 0.81^{b}	129.53 ± 0.40^{ab}
T5	9.13 ± 0.15^{a}	22.20 ± 0.72^{bc}	127.22 ± 1.56^{abc}
T6	9.33 ± 0.17^{a}	28.12 ± 1.52^{a}	125.90 ± 2.07^{bc}
T7	9.40 ± 0.13^{a}	29.53 ± 0.81^{a}	127.20 ± 0.99^{abc}
T8	9.17 ± 0.13^{a}	21.52 ± 0.42^{c}	126.38 ± 2.11^{bc}

Means bearing different superscript within the same column differ significantly at $P \leq 0.05$

Table 2. Mean (±SE) protein, BUN and creatinine levels of broiler chicks

GROUPS	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)	A:G RATIO	CREATININE (mg/dl)	BUN (mg/dl)
T1	4.12 ± 0.17^{a}	1.74 ± 0.06^{a}	2.37 ± 0.14	0.75 ± 0.04	0.33 ± 0.03^{c}	2.97 ± 0.42^{b}
T2	3.05 ± 0.14^{c}	1.37 ± 0.09^{b}	1.69 ± 0.16	0.86 ± 0.12	0.52 ± 0.04^{a}	5.66 ± 0.26^{a}
T3	3.89 ± 0.38^{ab}	1.73 ± 0.05^{a}	2.16 ± 0.42	0.96 ± 0.17	0.33 ± 0.03^{bc}	3.10 ± 0.23^{b}
T4	4.05 ± 0.28^{ab}	1.79 ± 0.03^{a}	2.26 ± 0.30	0.86 ± 0.10	0.50 ± 0.04^{a}	2.78 ± 0.39^{b}
T5	3.43 ± 0.25^{abc}	1.77 ± 0.01^{a}	1.66 ± 0.25	1.19 ± 0.17	0.41 ± 0.03^{b}	2.67 ± 0.25^{b}
T6	4.20 ± 0.15^{a}	1.73 ± 0.08^{a}	2.46 ± 0.22	0.76 ± 0.12	0.30 ± 0.01^{c}	3.16 ± 0.23^{b}

T7	3.29 ± 0.19^{bc}	1.69 ± 0.05^{a}	1.60 ± 0.23	1.19 ± 0.20	0.33 ± 0.01^{bc}	2.73 ± 0.36^{b}
T8	3.90 ± 0.29^{ab}	1.80 ± 0.07^{a}	2.10 ± 0.33	1.00 ± 0.19	0.38 ± 0.02^{bc}	2.79 ± 0.23^{b}

Means bearing different superscript within the same column differ significantly at $P \leq 0.05$

Mean (±SE) AST levels of all treatment birds recorded at weekly intervals are presented in Table 3. Mean (±SE) ALT levels of all treatment birds recorded at weekly intervals are presented in Table 4.

Table 3. Mean (±SE) AST (U/L) levels of broiler chicks

GROUPS	7th DAY	14th DAY	21st DAY
T1	189.63 ± 4.80^{bc}	199.36 ± 5.80^{b}	197.08 ± 2.83^{b}
T2	253.45 ± 9.24^{a}	263.33 ± 15.85^{a}	255.97 ± 13.85^{a}
T3	195.26 ± 6.53^{bc}	207.37 ± 5.69^{b}	192.57 ± 5.48^{b}
T4	181.36 ± 7.63^{c}	212.82 ± 10.53^{b}	189.18 ± 6.29^{b}
T5	202.67 ± 6.46^{bc}	204.25 ± 14.95^{b}	190.97 ± 6.67^{b}
T6	194.12 ± 6.27^{bc}	222.33 ± 11.07^{b}	199.33 ± 5.09^{b}
T7	196.42 ± 8.49^{bc}	208.53 ± 9.62^{b}	201.40 ± 5.86^{b}
T8	204.67 ± 5.37^{b}	204.60 ± 12.66^{b}	196.48 ± 6.18^{b}

Means bearing different superscript within the same column differ significantly at $P \leq 0.05$

Table 4. Mean (±SE) ALT (U/L) levels of broiler chicks

GROUPS	7th DAY	14th DAY	21st DAY
T1	6.10 ± 0.58^{bc}	4.31 ± 0.20^{bcd}	4.80 ± 0.32^{bc}
T2	10.76 ± 0.50^{a}	8.71 ± 0.50^{a}	11.91 ± 0.36^{a}
T3	6.04 ± 0.32^{bc}	3.91 ± 0.26^{cd}	4.35 ± 0.31^{bc}
T4	6.65 ± 0.77^{b}	5.41 ± 0.56^{b}	4.53 ± 0.40^{bc}
T5	4.41 ± 0.60^{c}	5.09 ± 0.19^{bc}	4.07 ± 0.50^{bc}
T6	5.64 ± 0.59^{bc}	3.43 ± 0.21^{d}	3.63 ± 0.36^{c}
T7	5.34 ± 0.65^{bc}	4.06 ± 0.29^{cd}	5.29 ± 0.40^{b}
T8	5.25 ± 0.53^{bc}	4.62 ± 0.54^{bc}	4.34 ± 0.65^{bc}

Means bearing different superscript within the same column differ significantly at $P \leq 0.05$

DISCUSSION

A significant reduction in the Hb concentration and VPRC values were noticed in T2 group compared to T1 group. The deleterious effect of AFB1 at 100 ppb in this study could be due to inhibition of protein synthesis, defective absorption of iron and haematopoesis suppression (Abeena *et al*., 2015). Group T3 did not reveal any deleterious effect on the Hb concentration. Jagetia *et al*. (2006) observed the protective effect of *A. marmelos* extract on reduction of Hb induced by radiation in Swiss albino mice and this could be attributed to its anti-oxidant potential. Abhishek *et al*. (2010) stated that *A. paniculata* prevents lipid peroxidation of RBCs and its oxidative damage by inhibiting binding of toxic metabolites to DNA. The protective effect of plant materials could be also due to chelating properties of flavonoids which drastically decreased the presence of unbound serum iron. The reduced TLC in T2 group in the present study could be attributed to the depressing effect of AFB1

on the haematopoietic cells (Rathod *et al*., 2017). The effect of both plant materials at concentrations of 0.10 per cent and 0.20 per cent individually or in combination at 0.10 per cent each were similar. Pratheepa *et al*. (2010) identified the ability of *A. marmelos* to induce lymphopoiesis and to increase WBC count. Abhishek *et al*. (2010) observed the ability of *A. paniculata* to induce the production of haematopoietic stimulator and haematopoiesis. The improvement in TLC observed in this study could be due to flavonoids present in the plants, which showed potent inhibition of collagen, arachidonic acid, thrombin and platelet activation factors (Abhishek *et al*., 2010) and drastically decreased the presence of unbound serum iron.

Total protein and albumin were signifcantly lowered in T2 group compared with T1. Reduced feed intake, inactivation of biosynthetic enzymes and impaired protein synthesis induced by AF lead to hypoproteinaemia and hypoalbuminaemia. Hypoproteinaemia and hypoalbuminaemia observed in T2 in the present experiment could be due to inhibition of protein synthesis caused by AFB1. Serum total protein of broiler chicks of T3, T4, T5, T6, T7 and T8 were higher than T2. Birds of T2 group fed with 100 ppb of AF showed significantly increased BUN values compared with T1. Significantly increased BUN values due to AF has been previously reported by Gounalan (2005) and attributed to kidney damage caused by the AF (Jayaramu *et al*., 2012). The beneficial effect of these plant materials in this present study could be due to the anti-oxidant potential of their active components (Aneesh *et al*., 2018a; Aneesh *et al*., 2018b). Creatinine levels were significantly increased in T2 group compared to T1. Similar results were previously reported George (2007) and attributed to identified nephrotoxic action of AF. Creatinine levels of T6, T7 and T8 were statistically similar to T1. Kuttan and Sabu (2004) identified the potential of *A. marmelos* extract to reduce both oxidative stress and antioxidant levels. Singh *et al*. (2009) interpreted that renoprotective action of *A. paniculata* due to antioxidant action of andrographolide, a major constituent of the plant (Gupta *et al*., 2015; Abhishek *et al*., 2010).

Levels of AST and ALT at seven, 14 and 21 days were significantly increased in T2 group of broilers fed with 100 ppb of AF compared to T1. Such effect on AST and ALT levels were previously identified by Dhanapal *et al*. (2014). The increased enzyme levels in T2 group of birds could be due to AF induced degeneration of hepatocytes and enzyme leakage to the blood stream. The protective effect of plant materials on the AST and ALT

value in this study could be attributed to hepatoprotective ability of plant materials.

CONCLUSION

The present study concluded the ameliorative effects of *A. paniculata* and *A. marmelos* plants on haematology and serum biochemistry among broiler chicken on aflatoxicosis. Different concentrations of these plant materials did not cause any significant variation in the effect. Both the plant materials at concentrations 0.10 per cent and 0.20 per cent were found to be equally effective. The combination of plant powders at 0.10 per cent each used without AF did not elicit any negative effect and its response was identical to control.

REFERENCES

Abeena, B., George, A.J., Mohammed Shejir, R., Nair, N.D. and Manomohan, C.B. 2015. Haematobiochemical changes of layer chicken in experimental aflatoxicosis. *Indian Vet.J.* 92:84-86.

Abhishek, N., Tiwari, S.K. and Alok, L. 2010. Biological activities of Kalmeg (*Andrographis paniculata* Nees) and its active principles- a review. *Indian J. Nat. Prod. Resour.* 1:125-135.

Aneesh, A., George, A.J., Kariyil, B.J., Krishna, D. and Abraham, M.J. 2018a. Phytochemical evaluation of the leaves of *Aegle marmeloes* L.(L.) - an important medicinal plant. *J. Trop. Agri.* 56:81-85.

Aneesh, A., George, A.J., Kariyil, B.J., Krishna, D. and Abraham, M.J. 2018b. Phytochemical evaluation of *Andrographis paniculata* L. (L.) *J. Pharmacognosy Phytochem.* 7:1949-1953.

Dhanapal, S.K., Rao, S., Govindaraju, P.K.P., Hukkeri, R. and Mathesh, K. 2014. Ameliorative efficacy of citrus fruit oil in aflatoxicosis in broilers: a growth and biochemical study. *Turkish J. Vet. Anim. Sci.* 38:207-211.

George, A.J. 2007. Effect of feeding sublethal doses of aflatoxin and ochratoxin in broiler chicken. PhD. thesis, Tamilnadu Veterinary and Animal Sciences University, Chennai, 78p.

Gounalan, S. 2005. Pathology of Newcastle disease virus and its interaction with mycotoxins in layer–type chicken. Ph.D. thesis, Tamil Nadu Veterinary and Animal Sciences University, Chennai, 98p.

Gupta, A.K., Verma, S. and Doshi, N. 2015. Phytochemical Analysis and Antioxidant Property of *Aegle marmelos* Extracts. *Int. J. Curr. Microbiol. App. Sci.* 4:826-830.

Jagetia, G.C., Venkatesh, P., Archana, P., Krishnanand, B.R. and Baliga, M.S. 2006. Effects of *Aegle marmelos* (L.) Correa on the peripheral blood and small intestine of mice exposed to gamma radiation. *J. Environ. Pathol. Toxicol. Oncol*. 25:611-24.

Jayaramu G.M., Satyanarayana, M.L., Ravikumar, P., Jagadeesh Sanganal, Paniraj, K.L. and Rao, S. 2012. Effect of Feeding Ochratoxin A and Citrinin toxins on Certain biochemical parameter in broiler chicken. *Global J. Biosci. Biotech.* 1:186-190.

Kuttan, R. and Sabu, M. C. 2004. Antidiabetic activity of *Aegle marmeloes* and its relationship with its antioxidant properties. *Indian J. Physiol. Pharmacol.* 48:81-88.

Pratheepa, V., Ramesh, S. and Sukumaran, N. 2010. Immunomodulatory effect of *Aegle marmelos* leaf extract on freshwater fish *Cyprinus carpio* infected by bacterial pathogen *Aeromonas hydrophila. Pharmaceutical Bio.* 48: 1224–1239.

Rathod, P., Gangadhar, K., Gangane, G. and Bhojane, N. 2017. Effect of aflatoxin on haematological and biochemical alteration in broilers. *Int. J. Sci. Environ. Technol.* 6: 824-831.

Shotwell, O.L., Hesseltine, C.W., Stubblefield, R.D. and Sorenson, W.G. 1966. Production of

aflatoxin on rice. *Appl. Microbiol.* 14: 425-428.

Singh, P., Srivastava, M.M. and Khemani, L.D. 2009. Renoprotective effects of *Andrographis*

paniculata (Burm. f.) Nees in rats. *Upsala J. Med. Sci.* 114:136-139.